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1

The controversial issue of the synaptic structure underlying pain modulation in the superficial dorsal horn of the spinal cord

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Abstract

At present, the occurrence of first modulation of pain transmission in the superficial dorsal horn, especially in the substantia gelatinosa (SG: lamina II), is firmly established. Nociceptive primary afferent central terminals (C-terminal) arising from nonmyelinated C-fibers and myelinated Aδ-fibers terminate in the SG and make synaptic glomeruli, in which they are centrally situated and surrounded by a number of dendrites and

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a few axonal terminals. One of the structural characteristics of C-terminals is indented, sinuous, or scalloped electron-dense axoplasm full of round synaptic vesicles (CI-terminal), while an other is larger roundish, electron-lucent axoplasm with many large dense core vesicles and mitochondria (CII- terminal). They are FRAP- and SP- or CGRP-positive and show intense capsaicin-sensitivity. Inhibitory interneurons containing GABA, glycine or opioids are exclusively present in the SG. They contact with C-terminals, forming synaptic glomeruli. However, the synaptic relationship between C-terminals and inhibitory interneurons is controversial, i.e., the postsynaptic contacts of inhibitory interneurons to C-terminals is not accepted. However, from previous reports concerning these synaptic structures, the presynaptic relationship of C-fibers to the inhibitory interneurons seem to be more enhanced, although species differences might be present.

Abbreviations

AChE: Acetylcholinesterase, ACTH: Adrenocorticotropic hormone, APP: Avian pancreatic polypeptide, CAL: Calretinin, CB: Calbindin-D28K, CCK: Cholecystokinin, CGRP: Calcitonin gene-related peptide, ChAT: Choline acetyltransferase, DOP: Dopamine, DYN: Dynorphin, ENK: Enkephalin, FRAP: Fluoride-resistant acid phosphatase, GABA: γ-Aminobutyric acid, GAL: Galanin, GLU: Glutamate (Glutamic acid), GluR1-4: AMPA-type glutamate receptor, GluR5-7: Kainate-type glutamate receptor, GLY: Glycine, HIS: Histamine, 5-HT: 5-Hydroxytryptamine (Serotonin), IB4: Isolectin B4, L-ENK: Leucine-enkephalin, M-ENK: Methionine-enkephalin, NA: Noradrenaline, NADPH-d: Nicotinamide adenine dinucleotide phosphate diaphorase, NMDAR: NMDA-type glutamate receptor, NP: Neurophysin, NPY: Neuropeptide Y, NT: Neurotensin, OXY: Oxytocin, $P2X_3$: ATP-gated receptor, Ret: GDNF receptor kinase, SOM: Somatostatin, SP: Substance P, TRH: Thyrotropin-releasing hormone, TrkA: NGF receptor tyrosine kinase, VIP: Vasoactive intestinal polypeptide, VR1: Vanilloid (Capsaicin) receptor 1 (TRPV1), VRL1: Vanilloid receptor-like 1 (TRPV2).

Introduction

There have been numerous studies on the fine structure of the synaptic relationship of the nociceptive primary afferent central terminals (C-terminal) to the inhibitory interneurons, using immunoelectron microscopic methods, from 1970 to date. The exact interrelationship between C-terminals and inhibitory interneurons is not yet clear, because the demonstration of pre- or postsynaptic contacts of C-terminals to inhibitory interneurons has been quite different for each investigator and each experimental animal. Thus, the ultrastructural relationships between C-terminals and inhibitory interneurons are reexamined based on previous reports. An anatomical basis underlying pain modulation in the superficial dorsal horn is a fundamental importance for understanding the complicated issue of pain transmission. Substantia gelatinosa (SG or Rolando substance) is a key area with anatomical, chemical and physiological properties, thereby functioning as a filter in nociceptive information [84]. Therefore, a brief description of the anatomical structures of the SG is helpful.

Interneurons in the superficial spinal dorsal horn

The cat spinal dorsal horn was classified into 6 regions (layers) according to the shape, size and the density of nerve cells by Rexed [64]. In this section, the architecture of the dorsal horn is briefly noted based on the three previous monographs [12,13,87]. Generally, the superficial dorsal horn contains lamina I (marginal zone), lamina II (substantia gelatinosa) and lamina III. Their boundaries are not necessarily clear. But the translucent appearance of the lamina II when unfixed spinal dorsal horn is observed by light microscopy discriminates it from the other layers. Since Cajal's observation, lamina II is divided into a thin outer and a broad inner (ventral) zone [64]. While the outer lamina II (IIo) is full of smaller cells and unmyelinated C fibers, the inner lamina II (IIi) includes both unmyelinated and small myelinated fiber endings demonstrated by electron microscopic and Golgi stainig methods [13]. Thus, the above anatomical evidence well coincides with the fact that the nociceptive information from the periphery conveyed through small primary afferent neurons reaches the spinal or trigeminal superficial dorsal horn, especially layer II, and the nociceptive input is believed to be first modulated there. Although there may be some problems simply classifying into cell types in lamina II with dendritic morphology, the most current criterion of the cell type based on the concise reviews [12,87] is convenient for the later description (Fig. 1). There are two main cell types in the lamina II; one is newly termed as Gobel's stalked cells (old name: Cajal's limiting cells) and the other Gobel's islet cells (old name: Cajal's central cells), slightly smaller (7~14 μm in dia.) than the former with scanty cytoplasm. The stalked cells, characteristically with short stalk-like spines, are localized in outer lamina II (IIo). This type of cells send axons into lamina I, thus called interlaminar interneurons. They are presumed to be driven by only noxious stimulus (nociceptive specific) or by both noxious and innocuous stimuli (wide dynamic range: WDR) [12]. The islet cells rostrocaudally project dendritic trees throughout the lamina II (80-150 μm in depth). The dendrites of islet cells in lamina IIo are thought to be nociceptive specific (low threshold: LT), whereas those in inner lamina II (IIi) are mechanoreceptive. These dendrites make synapses with other dendrites and axonal terminals. The axons of islet cells, corresponding to Golgi type II cells, are thought to end mainly in lamina II. Further, they are suggested to be inhibitory

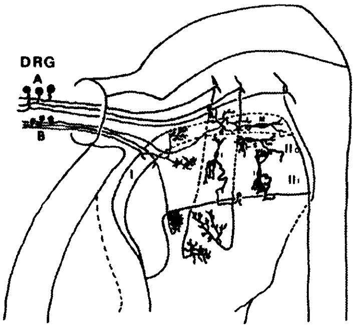

Figure 1. Schematic drawing of the central terminations of the large A type (A) and small B type (B) dorsal root ganglion neurons, and intrinsic neurons within the superficial dorsal horn. The fine nerve fibers (C-type) terminate in or near the superficial dorsal horn. DRG: dorsal root ganglion, I: islet cells (Cajal's central cells), M: marginal cell in lamina I (I), S: stalked cell in outer lamina II (IIo).

interneurons. Gobel et al. [26] reported the ultrastructures of islet and stalked cell processes in the IIo of the cat lumbar spinal dorsal horn by horseradish peroxidase (HRP) labeling method. The dendritic shafts and their spine heads of islet cells make asymmetric (postsynaptic) contacts with scalloped endings of the primary afferents [26]. They suggested that islet cells are GABAergic due to the similar morphology of the dendrites with GABAergic processes [26]. Other cell types (arboreal cells, II-III border cells, and spiny cells) are reported, but precise descriptions of these cells is not necessary in this chapter.

The projection of axons of some SG cells via Lissauer's tract through several segments and the cells in SG projecting to deeper laminae are confirmed [13]. In contrast, SG cells receive extensive dendrites from the cells in deeper laminae [87]. Moreover, an inhibitory descending pathway from supraspinal nuclei (rostral ventromedial medulla: nucleus raphe magnus) through the dorsolateral funiculus on the SG is stated [20]. Thus, it must be noted that the SG is not a closed system [87].

Chemical compounds found in the substantia gelatinosa

Chemical substances present in the SG are classified into main three sources; one originates from small primary afferent neurons, the second is synthesized within neurons in the SG (intrinsic SG cells), and the third is of supraspinal origin. While compounds originating in the SG are seen in intrinsic cells and their processes, the other two are seen in the terminals in the SG. Many compounds presumably participating in nociception in the SG of the spinal dorsal horn and spinal trigeminal nucleus (medulla) are summarized in

Table 1; that is, acetylcholinesterase (AChE), choline acetyltransferase ChAT), dynorphin, enkephalin, γ-aminobutyric acid (GABA), glutamate (glutamic acid), asparate, galanin, neurotensin, neuropeptide Y, serotonin (5-HT), somatostatin, substance P (SP), thyrotropin-relasing hormone (TRH), and vasoactive intestinal polypeptide (VIP) [87]. In addition, cholecystokinin [24], methionine-enkaphalin [24], oxytocin [24], neurophysin [24], adrenocorticotropin [24], avian pancreatic polypeptide [41], leucine-enkephalin [73], calcitonin gene-related peptide (CGRP) [21,23,43,75,82,86], fluoride-resistant acid phosphatase (FRAP) [45], nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d, an enzyme identical to nitric oxide synthase, NOS) [71], calcium binding proteins calbindin-D28K and calretinin [1,71], isolectin B4 (IB4) [6,55,77], nerve growth factor (NGF) receptor tyrosine kinase (TrkA) [55], glial cell line-derived neurotrophic factor, GDNF receptor kinase (Ret) [55], ATP-gated receptor ($P2X_3$) [27], vanilloid receptor (capsaicin receptor: VR1: TRPV1) [11,27], vanilloid receptor like-1 (VRL1: TRPV2) [48], glycine [62], and glutamate receptors [51,81,89]. Thus, the presence of numerous compounds in the SG reflects the complicated nociceptive transmission and modulation.

Table 1. Chemical compounds putatively participating in nociceptive transmission identified in SG and primary sensory neurons.

Compounds		DRG or TG cells	substantia gelatinosa (SG)		References
			cells	nerve fibers or terminals	
peptides	SP	•	•	•	73, 86, 87
	CGRP	•		•	43, 86
	VIP	•			87
	CCK	•	•		87
	OXY	•			24
	NP			•	24
	ACTH			•	24
	APP		•	•	87
	GAL	•	•		87
	NT		•	•	87
	NPY		•		87
	SOM	•	•	•	41, 87
	TRH		•	•	87

Table 1. Continued

	ENK	●	●		87
opioid	L-ENK	●	●	●	73
peptides	M-ENK			●	24, 43
	DYN	●	●		87
lectin	IB4	●		●	6, 11, 55, 77
	GABA	●	●	●	57, 76, 87
amino acids	GLU	●	●	●	87
and their	*5-HT			●	87
derivatives	GLY		●	●	62, 87
	HIS			●	87
catechol	*DOP			●	87
amine	*NA			●	87
Ca-binding	CB	●	●	●	49, 71
protein	CAL		●	●	71
	FRAP	●	●	●	45
enzymes	NADPH-d	●	●	●	1, 71
	AChE(ChAT)	●	●	●	87
glutamate	NMDA-type		●	●	51, 81, 89
receptors	GluR1-4(AMPA)	●	●	●	51, 81
	GluR5-7(kainate)		●	●	51, 81
	TrKA	●		●	55
other	Ret	●		●	55
receptors	$P2X_3$	●		●	27
	VR1	●		●	11, 27
	VRL1	●	●	●	11, 48

* supraspinal origin
● identified areas

Characteristic ultrastructures of central terminals of nociceptive primary afferent neurons in the substantia gelatinosa

Since Melzack and Wall [54] proposed the gate control theory that "the substantia gelatinosa functions as a gate control system that modulates the afferent patterns before they influence the T (projection) cells", the survey of ultrastructural details has been directed to the SG, as in the case for studies of the physiology and pharmacology. Most notably, Gobel [25] demonstrated the fine structures of the synaptic glomeruli in the SG of the cat spinal trigeminal nucleus caudalis. He emphasized the presence of many synaptic glomeruli in the SG. The outer glomerulus consists of many small dendritic shafts and their spines, and centrally situated are small axonal endings (P endings) with a dark, long sinuous, or scalloped contour closely packed with spherical synaptic vesicles and clustered mitochondria. The above dark central nerve endings found exclusively in the SG were suggested to be central terminals of the small dark trigeminal ganglion cells, probably playing roles in thermal and painful transmissions [25]. The nociceptive information was postulated to be regulated within the internal circuit of the SG glomeruli [25]. Similar fine structures of central terminals (C-terminal) were reported in the rat [16,65,68], monkeys [46,47,61] and mice [33,39]. The common features of the central terminals of the nociceptive primary afferents show a dark, irregular contour (thin slender, sinuous or scalloped) full of clear synaptic vesicles, surrounded by many dendrites and a few axonal terminals. In particular, the synaptic glomeruli in SG of the rat were classified into two types according to the electron density, shape of outline, number of mitochondria and compactness and diameter of synaptic vesicles in C-terminals [65]. Of these, type I glomerulus has an indented, centrally located dark sinuous or scalloped C-terminal (CI) with a few mitochondria and packed clear synaptic vesicles of various sizes (52-65 nm dia.), and is rich in the dorsal lamina II (IIo), whereas type II have a light and larger C-terminal (CII) of roundish contour with less packed synaptic vesicles of uniform size (47-57 nm dia.), more mitochondria and sometimes neurofilaments, and are predominantly seen in the ventral lamina II (IIi). Ribeiro-da-Silva and Coimbra suggested that CI-terminals originate from unmyelinated primary afferents and CII-terminals directly or indirectly (recurrent) from myelinated primary afferents. Later, Coimbra et al. [15] confirmed these terminals as being derived from primary afferents by their degeneration after dorsal root rhizotomy. In addition, since neonatal capsaicin treatment caused a 93 % reduction of CI-terminals in the SG of adult treated rats, they are clearly of unmyelinated C-fiber origin [66]. Réthlyi and Szentágothai [63] reported the presence of large dark sinusoid axon terminals (DSA) at the center of the synaptic glomeruli in the SG of the kitten lumbosacral spinal cord. These DSA terminals were suggested to be ramifications of the dorsally directed short axons of the unknown pyramidal neurons, due to their intact appearance 2 days after dorsal root ganglionectomy. However, their DSA terminals apparently correspond to C-terminals of the primary afferent from the criteria of the characteristic fine structures described above. Probably, DSA terminals did not show degenerations because of the short time after ganglionectomy.

Immunocytochemical characterization of the synaptic glomeruli in the substantia gelatinosa

Earlier studies on the chemical compounds (especially substance P as a strong candidate for neurotransmitters of the primary afferents) using their antibodies were examinations of the distribution of such compounds in the superficial dorsal horn and primary afferent neurons of several mammals [14,18,19,40,41,58]. SP was demonstrated in the primary afferent C-terminals [14,18,19,40,41,58] and their synaptic vesicles [14,18,58], but the synaptic architecture of C-terminals, surrounding dendrites and axonal terminals, was not clear. Initial electron microscopic study showed the SP-immunoreactive (ir) terminals making axoaxonic, axodendritic and axosomatic synapses in the superficial dorsal horn of the rat lumbar spinal cord [5]. SP-ir deposits were mainly seen in large granular synaptic vesicles (LGV, 70-110 nm dia.). Since some SP-ir fibers were present despite the dorsal root section and ipsilateral hemi spinal cord section, they were suggested to be SP-ir interneurons in the superficial dorsal horn. However, the particular structure of C-terminals described above, i.e., sinuous, indented, and scalloped outlines, was not clear from the previous report. Long slender, sinuous or scalloped SP-ir terminals containing reaction products in LGV were demonstrated at the center of the glomeruli in SG of the rat spinal trigeminal nucleus [60]. The SP-ir terminals making axosomatic contacts were also seen in the lamina IIo [60]. A well-known primary afferent marker, CGRP (calcitonin gene-related peptide)-ir terminals were reported to be located in the lamina I and outer lamina II of the lumbar spinal dorsal horn of the monkey [9,10] and rat [53]. The outline of CGRP-ir terminals seems to be different from that of SP-ir, although coexistence of CGRP and SP in the primary afferent neurons and depletion of CGRP and SP from these tissues by capsaicin treatment were both reported [22]. SP-ir fine structures of C-terminals in the superficial dorsal horn of both spinal and lower brain stem of rats were examined in detail by Ribeiro-da-Silva et al. [70]. Approximate 14 % of CI-terminals in type I synaptic glomeruli showed SP-ir. They were classified into two types, light and dark (packed clear synaptic vesicles, large dense core vesicles >3, synaptic sites >2) owing to the electron density of the axoplasm. Twenty five per cent of them made synaptic glomeruli in the ventral SG. The number of dark-type CI-terminals was severely diminished by neonatal capsaicin treatment. Therefore, the light- and dark-type CI-terminals were considered to be distinct; the former may be derived from SP-ir intrinsic interneuron or supraspinal, whereas the latter is of primary sensory origin [70]. In fact, SP-ir neurons descending to the spinal dorsal horn were demonstrated in the brain stem nuclei [67].

Synaptic correlations between nociceptive primary afferent C-terminals and inhibitory axonal terminals or dendrites in the substantia gelatinosa

1) Opioid synapses: Despite the opiate receptors on terminals of primary afferent neurons, ultrastructural correlations (axo-axonal synapses) have not been confirmed in the SG [17,74]. Hence, nonsynaptic release of opiates was raised [17]. Although primary afferent fibers are likely to be under presynaptic inhibition, Enk-ir dendrites forming presynapses to C-terminals have not been found [52]. Fifty per cent of Enk-ir neurons were demonstrated also as SP-ir in the rat SG [69]. These endings storing both SP and Enk were speculated to be interneuronal stalked cells. The SP-ir or -negative CI-terminals never showed postsynapses to Enk-ir endings [69]. Moreover, it was demonstrated that SP-ir axon terminals made presynaptic contacts with Enk-ir axon or dendrites in the superficial dorsal horn of the rat cervical spinal cord [44]. Apparently, the SP-immunonegative CI-terminal was shown to make contact with postsynaptic Enk-ir dendrite in this report.

2) GABAergic synapses: As shown in Table 1, GABA is predominantly seen in intrinsic interneurons (29 % of islet cells, [80]), axonal terminals and dendrites in the superficial dorsal horn. $GABA_B$ receptor binding sites on presynaptic terminals were detected in large numbers in lamina IIi of the rat cervical spinal cord by means of the ^{3}H-GABA labeling method [59]. $GABA_B$ binding sites reduced by approximately 50 % in SG after neonatal capsaicin treatment (50 mg/kg), and thus the major $GABA_B$ receptor sites were suggested to be on C and Aδ fibers of the primary afferents [59]. Thirty four per cent (29/85) of the vesicle-containing dendrites and 79 % (26/33) of the axons were found to be GABA-ir [79]. Eight of the immunoreactive axons and 5 of the dendrites were presynaptic to the central terminal (CI), suggesting presynaptic GABA release at dendrodendritic and dendroaxonic synapses in the lamina II. In addition, nine of the immunoreactive profiles of vesicle-containing dendrites were postsynaptic to CI terminals. GABAergic vesicle-containing dendrites postsynaptic to primary afferent terminals were also present in SG of feline spinal trigeminal nucleus caudalis [2]. All three types of primary afferent terminals ((DSV (dense sinusoid axon terminals) and RSV (regular synaptic vesicle terminals) appear to correspond to CI-terminal and RSV and LDCV (large dense core vesicle terminals) corresponding to CII-terminals in the rodent)) were reported to be presynaptic to GABA-ir profiles in the superficial dorsal horn of the monkey lumbar spinal cord [8]. Moreover, CGRP containing central terminals originated from Aδ and C primary afferents in the superficial dorsal horn of the monkey lumbar spinal cord were presynaptic to GABAergic vesicle-containing dendrites, indicating a poor probability of presynaptic inhibition of nociceptive information by GABA [29]. Electrophysiologically identified nociceptive C fibers within the dorsal root were labeled with HRP and their terminal boutons with apposed neuropiles in lamina II of the monkey spinal dorsal horn were immunoreacted with SP, CGRP and GABA [3]. As a result, C-terminals including large dense core vesicles immunoreactive to CGRP were never postsynaptic to GABAergic profiles. Those CGRP-ir C-terminals in lamina II of the monkey spinal dorsal horn did not show glomerular type (nonglomerular), and therefore were considered to be not Aδ but C-fibers [3]. It should be also emphasized that some of the GABAergic dendrites are clearly postsynaptic to the primary afferent central terminals. On the other hand, three types of GABA-ir elements in the SG of chicken spinal dorsal horn were reported [4,72]; GABA-ir axon terminal containing only clear synaptic vesicles presynaptic to SP-ir C-terminals, vesicle-containing dendrites (VCD) containing both clear vesicles and dense core vesicles making reciprocal synapses to SP-ir C-terminals, and GABA-ir dendrites postsynaptic to SP-ir C-terminals. From the latter two cases, the feed back mechanism (disinhibition) of nociceptive transmission was suggested within the intrinsic short circuit. However, it is important to note that the peroxidase anti peroxidase (PAP) reaction frequently hinders the precise synaptic relationships, as the authors pointed out [72]. In fact, there seem to be more postsynaptic relationships of GABA-ir elements to SP-ir C-terminals from their presented figures. Out of 15 CI-terminals presynapsing to GABAergic profiles, most of them (11/15) made presynaptic contacts with GABA-ir vesicle containing dendrites, whereas CII-terminals were more likely to be postsynaptic to GABAergic profiles [7]. One third of the GABA-ir dendrites also contained NOS [7]. Twenty six per cent of CII-terminals made presynaptic contacts with NOS-positive dendrites [7]. Although the function of NO remained unclear, presynaptic inhibition was suggested to have a minor role in modulation of nociception, at least in the rodent superficial dorsal horn [7]. Localization of glutamate (Glu) and GABA in the glomeruli of SG in the dorsal horn of the feline trigeminal pars caudalis and medullary dorsal horn was studied by double immunogold labeling method [42]. Confirming the existence of GABAergic profiles postsynaptic to C-terminals, presynaptic GABA axonal terminals to Glu-ir C-terminals was inferred [42]. Based on the synaptic vesicle accumulation at the presynaptic site and intense electron density at the postsynaptic site, C-terminals are more likely presynaptic to GABA-ir profiles from these figures. GABA-ir peripheral axons in type I glomeruli in the superficial dorsal horn of the rat lumbar spinal cord were not glycine-ir, whereas peripheral axons in type II glomeruli were immunoreactive to both transmitters [78]. Thus, it was speculated that peripheral profiles containing both GABA and glycine do not have a significant role of presynaptic inhibition in unmyelinated fibers, whereas peripheral profiles immunoreactive to both transmitters presynaptically control the information via CII-terminals [78]. However, the synaptic relationships between peripheral profiles and CI-terminals were not described in detail.

Capsaicin-sensitive CI- and CII-terminals were clearly seen in the SG of the mouse lumbar spinal cord (Fig. 2) [30,39]. Degenerating CI-terminals induced

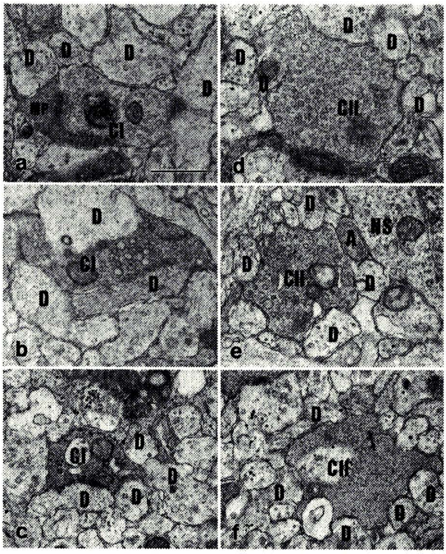

Figure 2. Degenerated central terminals in the substantia gelatinosa 12 h after capsaicin injection (50 mg/kg). **a**: A degenerating CI-terminal with a dense body. **b**: A degenerating CI-terminal showing many large vesicles and higher density axoplasm. **c**: A severely degenerated electron-dense CI-terminal showing degradation of axoplasmic organellae. **d**: A degenerating distorted CII-terminal with degraded synaptic vesicles and higher density axoplasm. **e**: A degenerating distorted CII-terminal with many degraded axoplasmic organellae and high density axoplasm. **f**: A severely degenerated CII-terminal showing a homogeneous electron-dense axoplasm with a degrading dense cored vesicle (arrow). A: axonal terminal, D: dendrite, MP: microglial process, NS: neuronal soma. Bar: 0.5 μm. (Modified from Hiura et al. [39]).

by capsaicin injection also demonstrated direct contact with interneuronal soma in the SG of the mouse dorsal horn (Figs. 3, 4) [31]. The CI- and CII-terminals showed FRAP-positive in the SG of the mouse spinal dorsal horn [32] and spinal trigeminal nucleus caudalis (STNC) [36]. Further, FRAP-positive CI-terminals formed direct synaptic contacts with interneuronal soma in the SG of the mouse STNC [36] and lumbar spinal dorsal horn [38]. CI- and CII- terminals making presynaptic appositions with surrounding dendrites were frequently seen in the SG of the mouse spinal cord [33]. GABA-ir dendrites were always postsynaptic to CI- or CII-terminals (Fig. 5) [33] and sometimes they directly presynapsed with GABAergic interneuronal soma (Fig. 6) [37]. The CI-terminals showing degeneration by capsaicin treatment were found to make synaptic contacts with GABA- or Met-Enk-ir dendrites and interneuronal soma in SG of the mouse lumbar dorsal horn (Fig. 7) [34]. SP-ir or both SP- and CGRP-ir CI-terminals showed synaptic contact with GABA-ir dendrites and

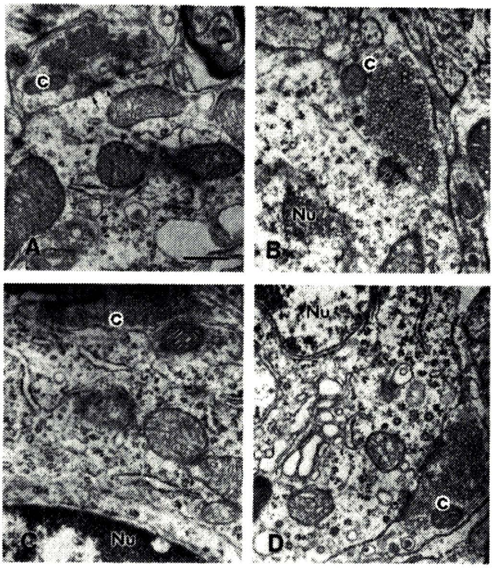

Figure 3. Synaptic contacts of nonglomerular CI-terminal with neuronal soma in the substantia gelatinosa of adult mice 2 h after vehicle injection (control). CI-terminlas, showing scalloped (**A**) or fusiform (**B**, **C**, **D**) contours with closely packed synaptic vesicles, make synaptic (**A**, **B**) or clear presynaptic (**D**) contacts with neuronal soma. A clear synaptic structure is not visible because the section was cut outside the synaptic structure (**C**). Arrows indicate synaptic site. c: CI-terminal, Nu: nucleus, Bar: 0.5 μm. (Modified from Hiura and Ishizuka [31]).

soma in SG of the lumbar spinal cord of the guinea pig [35]. Thus, the modulatory actions of nociceptive transmission in the SG were strongly suggested to occur exclusively by postsynaptic events [35]. Recently, it was clearly demonstrated that GABA- or glycine-immunoreactive dendrites were postsynaptic to SP-ir, sinuous CI-type terminals [85]. Therefore, SP-containing nociceptive fibers were predicted to inhibit pain information via GABA- or glycinergic short circuit [85].

The CI- and CII-terminals showed immunoreactivities to capsaicin receptor (VR1) in the superficial dorsal horn of the rat lumbar spinal cord [83]. As well as the demonstration that VR1- and SP-terminals were separate terminals, colocalization of VR1 and synaptophysin (presynaptic marker) was

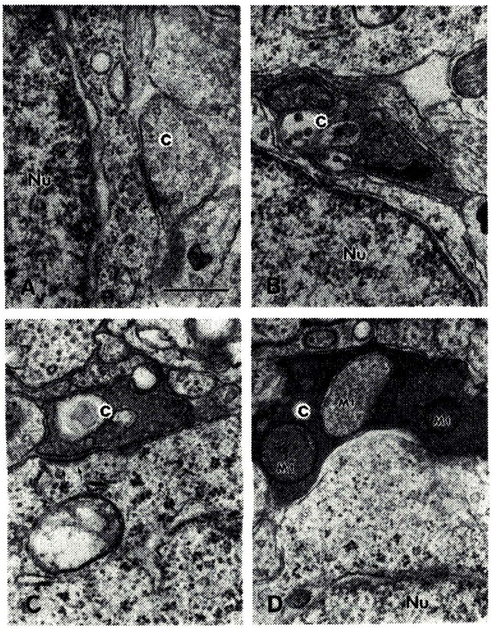

Figure 4. Nonglomerular CI-terminals showing various degree of degeneration on neuronal soma in the substantia gelatinosa of neonatal mice 2 h after capsaicin injection. **A**: A small roundish degenerating CI-terminal making presynaptic contact with neuronal soma. **B**: A degenerating CI-terminal showing dark axoplasm with degraded synaptic vesicles and mitochondria. **C**, **D**: Severely degenerated homogeneous highly electron-dense CI-terminlas with degenerated mitochondria and many vesicles. c: CI-terminal, Mt: mitochondria, Nu: nucleus, Bar: 0.5 μm. (Modified from Hiura and Ishizuka [31]).

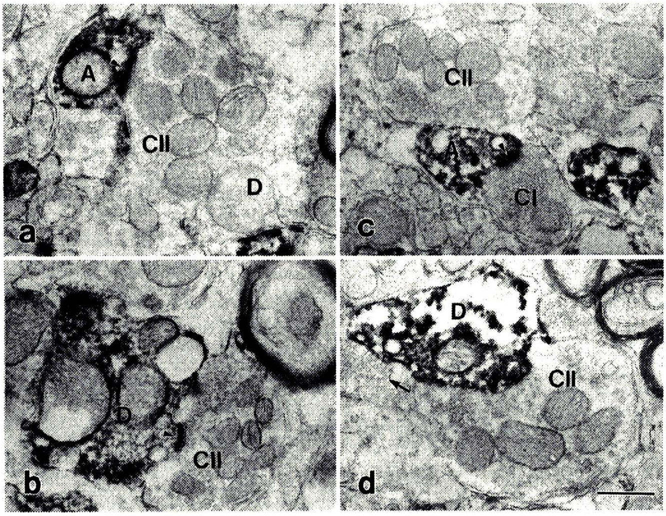

Figure 5. GABA-positive terminals showing contacts with CI (**c**)- or CII (**a-d**)-terminals. CII-terminals have many mitochondria beside synaptic vesicles. Arrowheads indicate thick postsynaptic membranes. A: axonal terminal, D: dendrite, Bar: 0.5 μm. (Modified from Hiura et al. [33]).

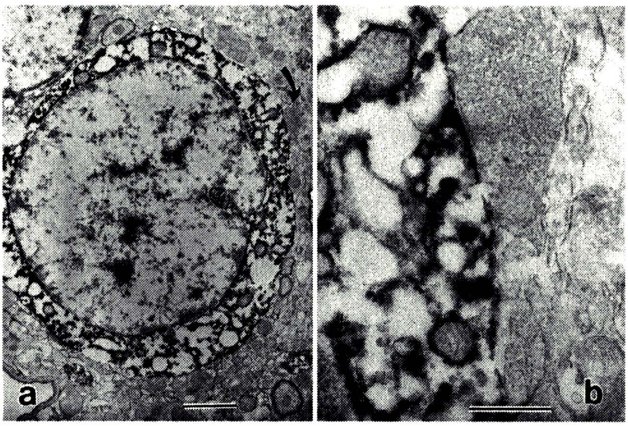

Figure 6. A typical CI-terminal (curved arrow) with closely packed clear synaptic vesicles contacting GABAergic somata. **a**: Low power electron micrograph. Bar: 2 μm. **b**: High power electron micrograph. Bar: 0.5 μm. (Modified from Hiura et al. [37]).

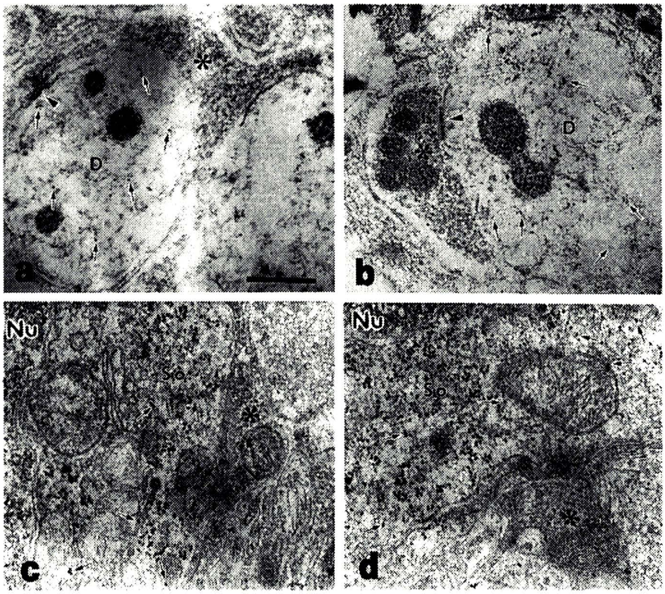

Figure 7. Synapses between degenerating CI-terminals 2 h after capsaicin injection and GABA- or Met-Enk-positive inhibitory interneurons in the substantia gelatinosa of the mouse lumbar spinal dorsal horn. **a**: A degenerating, sinuous CI-terminal (asterisk) making presynaptic contact with GABA-positive dendrite (D). Arrows indicate GABA-ir gold particles (15 nm). Arrow head indicates postsynaptic site. **b**: A degenerating, cap-like CI-terminal (asterisk) making presynaptic contact with Met-Enk-positive dendrite (D). Arrows indicate Met-Enk-ir gold particles (5 nm). Arrowhead indicates postsynaptic site. **c**: A degenerating, cap-like CI-terminal (asterisk) making contact with GABA-positive somata (So). Arrows indicate GABA-ir gold particles (15 nm). **d**: A severely degenerated, dark terminal (asterisk) making contact with Met-Enk-positive somata (So). Arrows indicate Met-Enk-ir gold particles (5 nm). GABA-ir gold particles also can be seen. Detection of the gold particles is difficult due to appearance of abundant glycogen particles in the cytoplasm (c,d). Nu: Nucleus, Bar: 0.5 μm. (Modified from Hiura et al. [34]).

demonstrated at 28 % and 11 % in lamina I and lamina II, respectively, by double immunostaining method [83]. It seems important to note that the majority of the VR1 terminals (CI-type) are presynaptic elements in the type I synaptic glomeruli.

Electrophysiologically, under the condition of a blockade of postsynaptic G-protein coupled $GABA_B$ receptors, presynaptic inhibition mediated by $GABA_B$ receptors located in primary-afferent Aδ-fiber terminals was reported in the SG of the rat lumbosacral spinal cord [88]. A decrease in $GABA_A$ receptor-mediated postsynaptic inhibition was recorded after partial peripheral nerve lesions; chronic constriction injury (CCI) and spared nerve injury (SNI) [56]. As a result, GABA-mediated postsynaptic and presynaptic inhibition of Aδ- and C-fiber nociceptors was suggested in the rat superficial dorsal horn [56]. In addition, it was demonstrated that C fiber input to the inhibitory islet cells triggered inhibition of SG neurons receiving excitation from nociceptive primary afferent C fibers in the SG of the rat spinal cord slices [50]. Together, the ultrastructural relationship between C-fibers and the inhibitory interneurons strongly suggests the regulation of pain transmission by nociceptive fibers themselves (i.e., feedforward inhibition) in the SG [31,34].

In conclusion, there is a possibility of the existence of presynaptic connections of primary afferent central terminals (C-terminal) to surrounding dendrites from intrinsic inhibitory neurons in the SG of the spinal cord and medullary dorsal horn of mammals. From the anatomical and electrophysiological evidence reported previously, the postsynaptic inhibitory short circuits in the SG should be stressed (Fig. 8). Studies combining skilful morphological and

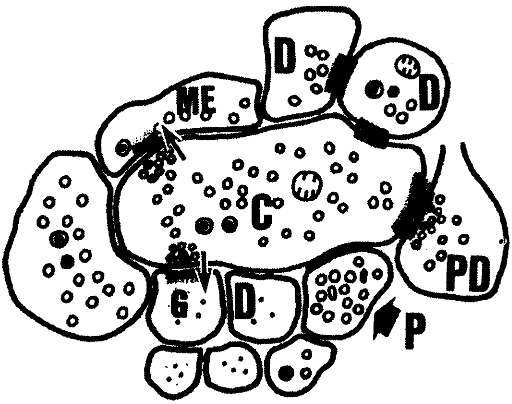

Figure 8. A schematic drawing on the synaptic relationship between central terminal (C) of the nociceptive primary afferent and inhibitory intrinsic neurons in the substantia gelatinosa of the mouse spinal dorsal horn. While C-terminals apparently form presynapses on GABA (G) - or Met-Enk (ME)-positive interneurons, their postsynaptic contacts to inhibitory interneurons can scarcely be seen in the SG of mice. This may indicate that C-terminals make presynapses with inhibitory interneurons in the substantia gelatinosa, but receive presynaptic inputs of inhibitory neuronal processes in the deep layer of dorsal horn, where projection neurons are present. This synaptic relation needs to be investigated. Arrows represent the direction of synaptic information. D: Dendrites, P: Axonal ending forming symmetrical axoaxonic synapse, PD: Presynaptic dendrite.

electrophysiological techniques are required for the interpretation of controversial pain regulation in the SG of mammals. A recent interesting report suggested that nociceptive inputs from C-fibers (nociceptors) provoke repetitive action potentials in SG-GFP interneurons, i.e., tonic Cajal's central neurons (>80 % GABA-ir) located in the lamina IIo and characterized by green fluorescent protein (GFP), followed by GABAergic inhibitory action on other neurons of the superficial dorsal horn, using transgenic mice with dorsal horn cells expressing enhanced GFP [28].

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